REPRODUCTION IN PHORONOPSIS VIRIDIS. THE ANNUAL CYCLE IN THE GONADS, MATURATION AND FERTILIZATION OF THE OVUM

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The question of the development, maturation and fertilization of the gametes is one of the many aspects of the biology of the Phoronidea on which available information is very incomplete. There are two accounts of gonad development (Ikeda, 1903; de Selys Longchamps, 1907), but there have been no previous studies of the maturation and fertilization of the egg. A related problem concerns the possibility of protandry in species other than the three known to be hermaphrodite. In most cases studies have not been sufficiently detailed to allow for a decision between protandry and dioecism.

The following account is concerned with an apparently dioecious phoronid, *Phoronopsis viridis*. It deals with the cyclic changes in the gonads, the maturation and fertilization of the egg, and includes some observations, made incidental to the main body of work, which bear upon the question of protandry in this group.

Methods

Phoronopsis viridis was collected from a tidal mud flat, Elkhorn Slough, situated in Monterey County, California, for a period of over 12 months at intervals of approximately four weeks. Before examination individuals were removed from their tubes by breaking the latter with fine forceps. Specimens from each collection were preserved in Bouin's fixative. Individuals used in the study of the gonads and nephridia were sectioned using either the paraffin or freezing technique. Paraffin sections were stained with Harris' hematoxylin and counterstained with alcoholic eosin. Material to be cut as frozen sections was bulk stained in alum cochineal before embedding in gelatin.

Eggs were obtained from mature specimens of *Ph. viridis* by puncturing the body wall in the reproductive region and allowing the ova to fall out. Since these eggs were found to be already fertilized they were simply pipetted into bowls of fresh sea water and allowed to develop at approximately 13° C.

Ova to be sectioned were fixed in Carnoy's fluid, stained whole by the Feulgen technique and counterstained in fast green. They were placed in a drop of horse serum on a slide, and the slide supported above a 50:50 mixture of glacial acetic acid and full-strength formalin in a warm petri dish. Within a few minutes the horse serum coagulated, and a small block containing the ova could then be cut from the drop on the slide. This block was then dehydrated, cleared, using tertiary butyl

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alcohol, and imbedded in paraffin. Sections were cut at 5 μ and drawings of the sections were made with the aid of a camera lucida.

Whole mounts of ova stained in phosphotungstic acid hemotoxylin or by the Feulgen technique were also studied.

General Structure

Phoronopsis viridis was described by Hilton (1930) from specimens found at Moro Bay in southern California. The animals from Elkhorn Slough fit Hilton's description well with regard to those characteristics customarily used in the taxonomy of this group. These features are the general appearance, size, degree of spirality of the lophophore, number of tentacles and of longitudinal muscles and the position of the longitudinal nerve cord.

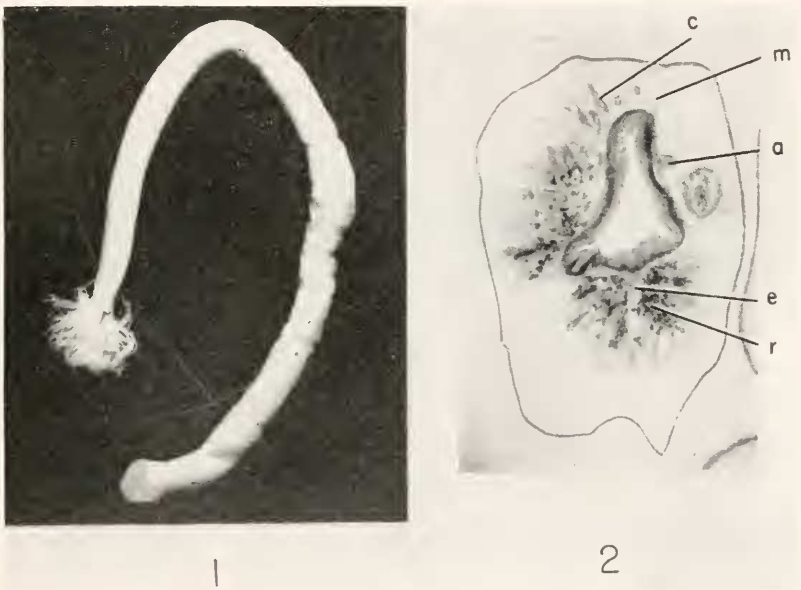

Figure 1. *Phoronopsis viridis.* The animal removed from the tube.

Figure 2. A cross-section of the body taken through the reproductive region. The reproductive tissue may be seen in the two anterior coelomic chambers. a—afferent vessel, c—capillary caecum, e—efferent vessel, m—mesentery, r—reproductive tissue.

Phoronopsis viridis is found in intertidal flats where the substratum is quite firm, consisting of a mixture of mud and sand. The animals inhabit tubes of a transparent secretion to which are cemented sand grains. The tubes, which are straight and occur vertically, vary in length from about 4 to 6 cm. and in diameter from 2 to 3.5 mm. The lower end tapers sharply in the last 3 or 4 mm. and is closed. The upper end of the tube is open and is usually flush with the surface of the mud. When the mud flat is covered with water the tentacles may project from the open end of the tube. The body is quite extensible and may extend 2 or 3 cm. beyond the end of the tube when the animal is not disturbed. The tube fits the animal tightly and it

is difficult to remove the latter intact. The general appearance of the animal out of its tube is shown in Figure 1.

Both mouth and anus open at the base of the coiled lophophore which bears the tentacles. The anus is borne on a papilla which is situated between the two arms of the lophophore, and the paired nephridia open on either side of this papilla near its base (Fig. 3). Below the lophophore there is a fold in the body wall forming the collar, and below this again is the tubular body which contains the digestive tract, the longitudinal blood vessels and the gonad. There are four longitudinal mesenteries which divide this part of the body cavity into four coelomic spaces. As can be seen from Figure 2 these mesenteries are not evenly spaced along the circumference of the body and consequently the left and right posterior chambers are considerably smaller than are the two anterior chambers. The afferent blood vessel lies within the right posterior chamber and the efferent vessel within the left anterior chamber

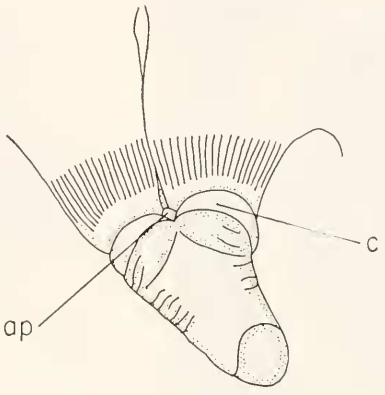

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FIGURE 3. The oral end of the animal, posterior view. ap—anal papilla, c—collar.

(Fig. 2). In the reproductive region of the body the efferent vessel gives rise to many short, blind branches, or capillary caeca (Fig. 2), and it is around these vessels that the gonads develop. The reproductive tissue is found only in the two anterior coelomic chambers, these areas being served by capillary caeca from the efferent vessel. The gonad is found in the aboral end of the body and may occupy from one- to two-thirds of the total length of the animal (Fig. 1). The body wall in this region is very thin and in season may become distended with gametes. The sexes are separate and may be easily distinguished during the breeding season, as the male gonad appears white and the female pink.

At times other than the breeding season a tissue known as the fat-body ("Fettkorper," Kowalevsky, 1867) or as vasoperitoneal tissue ("Vasoperitonealgewebe," Cori, 1939) is present around the capillary caeca. In life this tissue is jelly-like in consistency and transparent. Its structure, various inclusions and cycle of development are described below.

Annual Cycle in the Gonads

The developmental sequence in the gonad of *Phoronopsis viridis* is very similar to that described for *Phoronis ijimai* and *Phoronis australis* by Ikeda (1903) and for *Phoronis psammophila* by de Selys Longchamps (1907).

Female Gonad. The breeding season for *Ph. viridis* lasts throughout most of March and April. In the case of the female, the gonad in late February contains large numbers of full-sized ova, each about 60 μ in diameter. At this time most of the ova still possess the large germinal vesicle. They may be packed very closely and each is covered by a thin, squamous epithelium, one cell thick. The remnants of the vasoperitoneal tissue take the form of small patches or strands of granular cytoplasm between the masses of ova.

By about the middle of March many of the ova have become freed of the investing membrane. As this happens the germinal vesicle breaks down and the first maturation division begins. This division proceeds to first metaphase and stops (Fig. 4). The female nucleus remains at this stage until the egg is shed from the

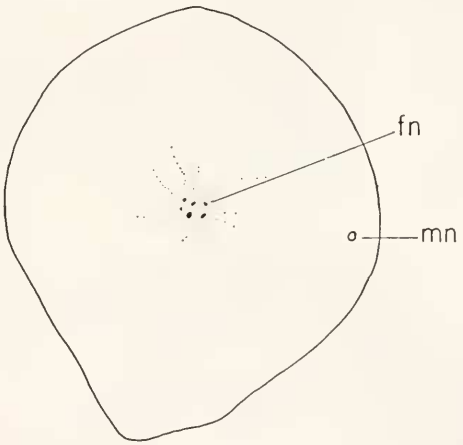

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Figure 4. Camera lucida drawing of an ovum from the body cavity. The female nucleus is in metaphase. From a female collected in March. fn—female nucleus, mn—male nucleus.

body. Once the ovum is freed of its investing epithelium it moves toward the periphery of the body and in the space between the outer surface of the gonad and the body wall the ripe ova collect. Occasionally an ovum will proceed to divide while still in the body cavity. Up to three or four such embryos have been found in one adult. Those found have always been blastulae. Similar embryos have been seen by de Selys Longchamps (1907) in *Phoronis mulleri* and *Phoronis sabatieri.*

Throughout the months of March and April large numbers of ova ripen and become free in the body cavity, where fertilization takes place. By the end of April most of the large ova have been spawned and there remain along some of the

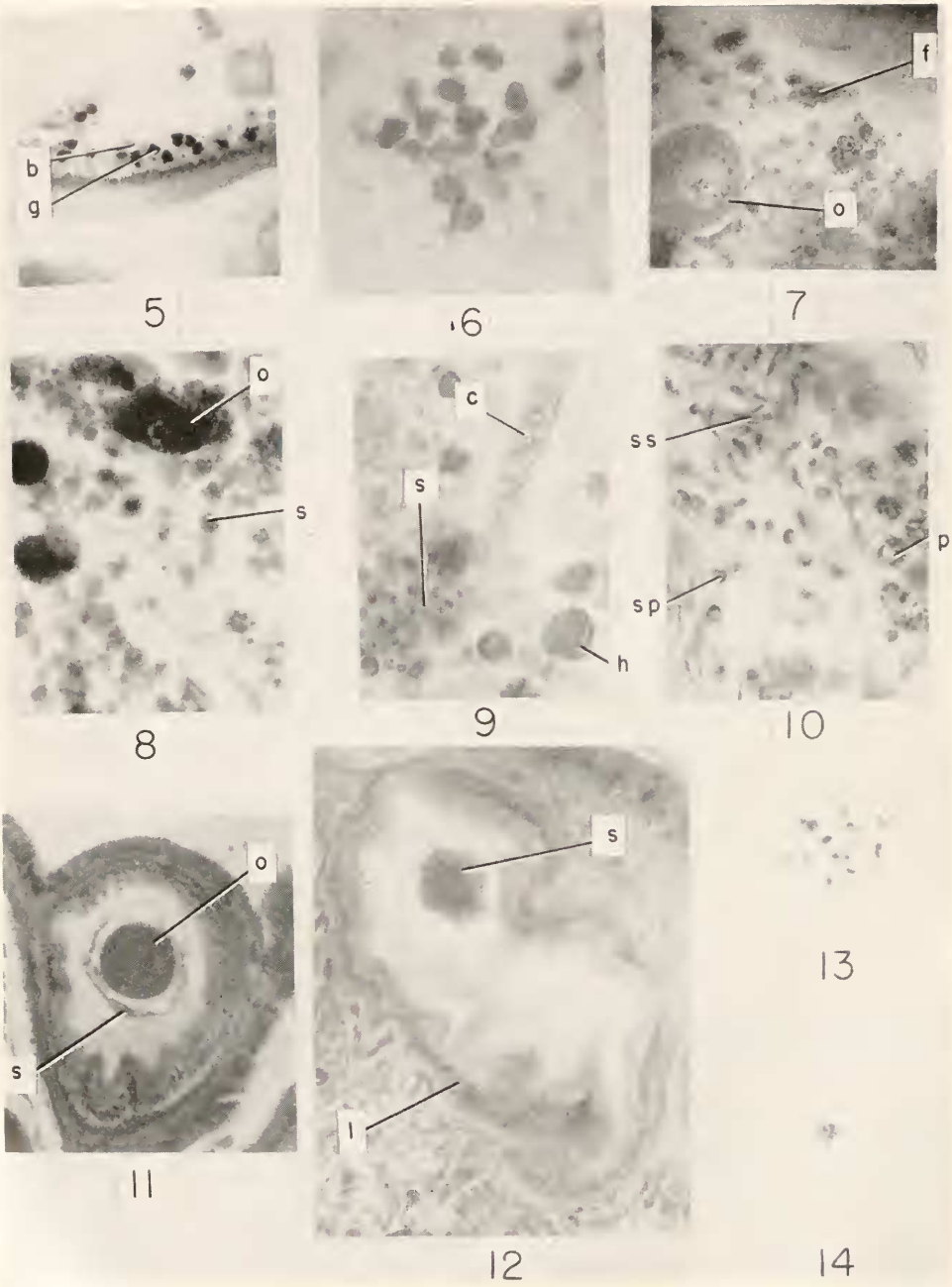

FIGURE 5. Opaque, non-staining granules, seen in a blood vessel. b—blood corpuscle, g—granule.

FIGURE 6. Dark green bodies, photographed from a smear of living tissue.

capillary caeca a considerable number of small ova, usually no more than 10 or 15 μ in diameter and frequently much smaller. These ova are presumed to be the forerunners of next year's crop of eggs which began their development before this year's crop was shed.

At the end of April it is possible to examine some of the capillaries while they are entirely free of reproductive tissue. The walls of such vessels have been found to consist of a single layer of small, flat cells. These cells are said to be a part of the mesodermal splanchnic epithelium (Cori, 1890) and the capillaries, therefore, to be merely spaces between sheets of splanchnic peritoneum. Radiating from the capillaries are strands of tissue containing occasional nuclei, separating clear unstained spaces which are presumably filled with fluid in life and represent the spaces left by the discharge of the ripe ova.

In the body cavity of *Ph. viridis* and particularly in the reproductive region, there are a number of kinds of cells or bodies which may be found free in the coelomic fluid, or contained within the vasoperitoneal tissue. Such elements are present only in small numbers in and around the gonad at the breeding season, but may be found in this region in greater abundance at other times of the year. They have not been studied in sufficient detail to make possible an opinion of their functional role. There are, however, three types of bodies which have been seen repeatedly in almost all animals studied and these are described below.

The most obvious and frequently the most abundant of these elements is a fusiform body about 15 μ long. It stains a uniform pink with eosin, contains no apparent nucleus, and the surface is often marked with faint longitudinal lines (Fig. 7). Similar bodies have attracted the attention of many persons working with the Phoronidea and it has been suggested by Pixel (1912) that they are granules of nitrogenous waste material because of a positive reaction to the murexide test. As far as I know no other attempt has been made to determine their chemical nature.

In some, but not all of the animals studied there appeared groups of small, opaque granules (Fig. 5), which do not stain with either hematoxylin or eosin and which retain their color throughout fixation and dehydration. Their color under transmitted light is gold; they vary in size from 2 to 8 μ and have been found in the

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FIGURE 7. Fusiform, eosinophilic body, seen in a female animal. f—fusiform body, o—ovum.

FIGURE 8. Non-granular, eosinophilic spheres in the vasoperitoneal tissue. o—ovum, s—eosinophilic sphere.

FIGURE 9. Coarsely granular, hematophilic body in the vasoperitoneal tissue. c—capillary caecum, h—hematophilic body, s—spermatocytes.

FIGURE 10. Section through the gonad of a male animal collected in February. p—primary spermatocyte, sp—spermatozoa, ss—secondary spermatocyte.

FIGURE 11. Section through the nephridial duct of a female animal collected in March. o—ovum, s—spermatozoa.

FIGURE 12. Section through the nephridial duct of a male animal collected in March. l—columnar lining of duct, s—spermatozoa.

FIGURE 13. Aceto-carmine squash preparation of the metaphase nucleus of an ovum in the body cavity. There are 13 pairs of chromosomes.

FIGURE 14. Aceto-carmine squash preparation of the male nucleus found in an ovum in the body cavity.

blood vessels as well as in the body cavity. Similar granules have been described from *Phoronis psammophila* by Cori (1939), who believed them to be inactive and degenerate amoebocytes.

At any time of the year some individuals contain minute dark green bodies in the coelomic cavity (Fig. 6). These bodies are non-motile, occur singly or in groups of two or more, and may perhaps be algal cells.

Following spawning, the development of next year's ova continues and many new ova appear. The development of new ova is most rapid at the proximal ends of the capillary vessels, on the efferent vessel and on the walls of the blood sinus surrounding the digestive tract. The germ cells are at first small, roughly spherical and contain a few darkly staining granules. They are separated from the lumina of the capillaries by a single layer of flat cells and are covered by a similar layer. In the case of the lateral vessel these epithelial cells lie outside the muscular elements in the wall of the vessel. The reproductive cells appear to be produced continuously, so that any given length of capillary wall will bear a series of ova of different ages, the oldest lying farthest from the parent vessel.

As the new generation of ova is beginning to develop, the vasoperitoneal tissue reappears. As early as May the clear spaces left by the discharged ova begin to fill with a finely granular cytoplasm containing many eosinophilic spheres of various sizes (Fig. 8). The cytoplasm and granules appear to be contained within very large cells separated from one another by membranous walls containing the nuclei. These cells appear to vary in length from about 25 μ to 75 μ and in diameter from about 6 μ to 14 μ. In general they are arranged around the blood vessels so that the long axes of the cells are at right angles to and radiate from the long axis of the capillary. There may be exceptions to this pattern, but since the capillary caeca pass in a number of directions it is difficult to determine by means of sections the relationships of all the cells of the vasoperitoneal tissue. The size and arrangement of these cells is similar to that described for *Phoronis psammophila* by Cori (1939). The developing ova become imbedded in the extensive peritoneal tissue and push into it as they increase in size.

There are two main types of inclusions found only in the cells of the vasoperitoneal tissue. The more abundant of these is the above-mentioned non-granular sphere, varying in diameter from less than 1 μ to about 8 μ (Fig. 8). These spheres stain with eosin and come to fill most of the substance of the vasoperitoneal tissue. The other type of inclusion is a coarsely granular, roughly spherical body varying in diameter from about 5 to 15 μ. This type of body stains with hematoxylin and appears in greatest abundance in the late summer and fall (Fig. 9).

In July all the ova are small and are found chiefly around the proximal ends of the capillaries, close to the digestive tract. By November the reproductive tissue has spread along the blood vessels, covering them for most of their length. At this time the larger ova are about 35 μ long and many new ones are being formed. By January the ova have increased in size, the larger ones being about 50 μ long and usually somewhat flask-shaped. Each ovum is still surrounded by a single layer of flattened cells. The germinal vesicle is large and contains a diffuse chromatin network as well as a large nucleolus in the shape of a curved disc. In February the gonad is packed with many large ova, 50 to 60 μ in diameter, and there are still many small ones along some of the capillaries. By this time the vasoperitoneal

tissue has been almost eliminated; the inclusions found in this tissue in late summer and fall have disappeared and all that remains in the occasional spaces between ova are thin strands of tissue which probably represent the membranes of exhausted cells. It has been impossible to determine whether the developing ova penetrate into or between the cells of the vasoperitoneal tissue. Within the next month the ova ripen, are spawned, and the cycle of development of the gonads begins again.

Male gonad. Fully formed, active spermatozoa are found in male animals as early as December. The quantity of active male gametes increases throughout January, February and March. In March the aboral ends of male animals are usually a dense white due to the masses of spermatozoa inside. Spawning may take place in February, March and April.

In May, immediately after the end of the breeding season, there is apparently no reproductive tissue left. In contrast to the situation in the female, the tissue which in the male will give rise to next year's gametes does not appear until the breeding season is over and the present crop of spermatozoa shed. Later in May the vasoperitoneal tissue re-forms and the two types of inclusion described in the case of the female appear. At this time a few cells which are probably spermatocytes may be seen along some of the capillaries. By June the vasoperitoneal tissue is well formed and reproductive cells are abundant along the blood caeca. By August the quantity of reproductive tissue has increased considerably and throughout the fall months the proportion of generative tissue increases steadily as the extent of the vasoperitoneal tissue decreases.

The germ cells of the male arise in the walls of the blood vessels in the same manner as do those of the female. Spermatocytes can be distinguished readily in sections, but spermatogonia have never been satisfactorily identified. It seems probable that they resemble the primary spermatocytes. The latter are roughly spherical cells, about 3 μ in diameter, in which the nuclear material occupies most of the cell body. They are found immediately adjacent to the capillary walls, sometimes forming masses up to 20 cells deep. In some cases a single row of such cells along a capillary wall has been observed to be covered by a sheet of thin epithelial cells. No such epithelium has been seen in connection with larger masses of spermatocytes or their derivatives. The secondary spermatocytes are smaller, about 1 μ in diameter, and are typically found farther from the capillaries than the primary spermatocytes. The secondary spermatocyte consists almost entirely of nucleus and in some cases attempts were made to count the chromosomes. The highest number counted was 12. In December and throughout the breeding season the groups of spermatocytes become fringed with masses of fully formed spermatozoa. The latter appear first in pairs, often attached to one another at one end and for a part of their length (Fig. 10). Such pairs are usually distributed between the secondary spermatocytes and the single spermatozoa. During the breeding season clumps of spermatoza, aggregated so that the heads are together and the tails free, are frequently found in the body cavity of male individuals. When such clumps are placed in sea water they tend to disperse.

As in the female, the male gametes develop at the expense of the vasoperitoneal tissue. During the breeding season and immediately afterward the latter tissue is represented only by cytoplasmic strands radiating from the walls of the capillaries.

When spawning begins in the male there are many spermatocytes present, but by the end of the breeding season they have all disappeared and it is not clear whether they all develop to spermatozoa or whether some spermatocytes degenerate without reaching maturity. It is also possible that the small size of the spermatocytes may render them so inconspicuous in small numbers that under these conditions they appear to be absent. At any rate, a fairly careful study has failed to reveal any male reproductive tissue in the gonad immediately after the close of the breeding season.

It seems likely that both sexes spawn more than once during the breeding season. Sections of the gonad taken in March and April show areas depleted of gametes as well as areas filled with mature germ cells. The mid-body region of such animals is usually not filled with gametes and it seems probable that the eggs and spermatozoa from the depleted areas of the gonad have already been spawned. In the laboratory the animals usually spawn at night, emitting a slow stream of eggs or spermatozoa, but the quantity of material released under these conditions must certainly represent only a fraction of the total quantity produced.

Protandry

It has been suggested by a number of authors that some species of Phoronidea may be protandric, being first male and then female. It seems certain in the case of *Ph. viridis* that at any one breeding season an animal is either male or female. Moreover, from a study of the development of the female gonad it seems probable that a female at one breeding season will be a female at the next, as the ova for the following year are already present in small numbers. In the case of the male the evidence is inconclusive. The reproductive tissue disappears or else becomes exceedingly inconspicuous after spawning and once the season is over there is no way of recognizing a male except by the absence of ova.

If *Ph. viridis* were truly protandric, all individuals when they first reached sexual maturity would be males, and at some subsequent time would become females. If this were so it would be reasonable to expect that males might tend to be smaller than females. There is a considerable range of size among mature individuals of this species, but the length of life and rate of growth are unknown. Also it is well known that individuals may cast off the lophophore and tentacles when disturbed, and it is possible that constriction resulting in separation may occur in other parts of the body. Cases of fission of this type have been reported for *Phoronopsis albomaculata* (Gilchrist, 1907) and for *Phoronis ovalis* (Marcus, 1949). If this type of asexual reproduction does occur in *Ph. viridis*, then the length of the adult body may bear no direct relationship to the age of the animal. There has, however, been found no good evidence of asexual division in this species and hence measurements were made of the lengths of a number of male and female individuals to determine whether or not the sexes showed any significant difference in length.

These measurements were made throughout one breeding season (1949) on all animals removed intact from their tubes. A total of 169 animals was measured, of which 95 were males and 74 were females. The average length of males was 5.6 cm. and of females 6.75 cm. The standard deviation was 1.35 in the male group and 1.01 in the female group. The standard error of the difference between the means

of male and female populations (d/d) was calculated according to the formula in Simpson and Rose (1939), and was found to equal 0.545. When this value is referred to the tables of "t" the probability is found to be 0.6 (p value).

From this it is apparent that there is no significant difference in length between male and female individuals. The males are not significantly smaller than the females, as might be expected if the animals were protandric. This analysis does not, of course, prove that *Ph. viridis* is not protandric; it merely indicates that on the basis of length of body there is no evidence for protandry.

Spawning, Fertilization and Maturation of Ova

The gametes of *Ph. vididis* pass out of the body by way of the nephridia. There are two nephridia which open to the exterior on either side of the anal papilla. Each nephridium is roughly U-shaped; the longer arm of the U opens to the exterior by a small pore; the shorter arm terminates in a pair of long funnels. One funnel of each nephridium opens into the posterior coelom and one into the anterior coelom. The walls of the duct are lined with columnar epithelium bearing very long, fine cilia (Fig. 12). The cells lining the funnels are also columnar and ciliated, but are narrower and more densely packed. The nephridia extend through the collar region of the body and are about 0.5 mm. long.

Examination of living animals at the time of breeding reveals that the contents of the reproductive region of the body are in constant motion as a result of contractions of the muscles of the body wall in the mid-body and reproductive regions. It seems probable that these movements are instrumental in getting the ova, once freed of investing membranes, up into the oral end of the body near the funnels of the nephridia. The average body length is about 5 or 6 cm.; the ova mature some 3 cm. from the nephridial funnels and must in some manner be propelled this distance before spawning can take place.

Sections of male animals, preserved in March, at the height of the breeding season, show large numbers of spermatozoa in the nephridium (Fig. 12). The spermatozoa occur in concentrated, roughly spherical masses which are found in both arms of the nephridium.

Sections of female animals preserved in March show both ova and spermatozoa in the lumen of the nephridium and in the body cavity (Fig. 11). The spermatozoa in the female nephridium may occur in dense masses and in some cases an ovum may be seen passing through a loose mass of spermatozoa (Fig. 11). Similar masses have been found in the mid-body region of a female collected in January. During the months of February, March and April spermatozoa may be found dispersed throughout the reproductive region of the female. No male tissue has ever been found in an otherwise female animal and it is probable that the spermatozoa which appear in the body of the female in January or February have come from a male animal. It seems likely that the spermatozoa enter the body of the female through the nephridium and fertilize the ova as they become free in the coelom. A male nucleus can usually be seen in ova which are free in the coelom and in which the female nucleus is in first metaphase (Fig. 4), but has not been found in ova which are still covered by the investing epithelium and in which the germinal vesicle remains intact. The male nucleus is very small and has been identified only by means of the Feulgen technique.

Once the ova has passed, by natural or by artificial means, from the body cavity into sea water the egg nucleus becomes active. Aceto-carmine squash preparations of ova from the body cavity reveal 13 pairs of chromosomes (2 n complement) on the metaphase plate (Fig. 13). The male nucleus may be seen in the preparations, although the chromosomes are not distinguishable (Fig. 14). Ten minutes after release into sea water the chromosomes of the egg nucleus have passed from metaphase to anaphase. At fifteen minutes the first polar body is forming. Thirty minutes after liberation the chromosomes are in metaphase of the second maturation division which takes place near the surface of the egg, usually very close to the first polar body. As the second polar body is forming the first usually divides.

During the first thirty minutes after liberation, while the two maturation divisions are taking place, the male pronucleus remains inactive. It may be found in a variety

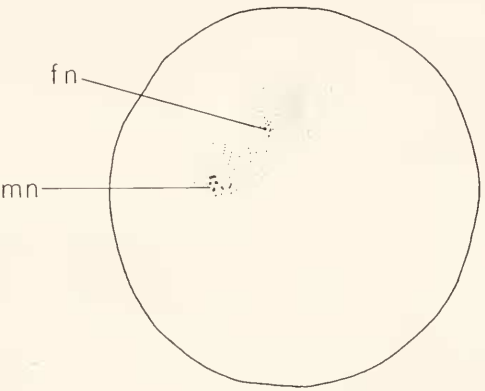

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FIGURE 15. Camera lucida drawing of an egg fixed forty minutes after liberation from the body cavity. Asters are forming around both male and female pronuclei prior to the formation of a fusion nucleus. fn—female nucleus, mn—male nucleus.

of positions within the egg, usually at some distance from the female pronucleus. Shortly after the formation of the second polar body the individual chromosomes of the male pronucleus become visible and an aster forms around them (Fig. 15). About forty-five minutes after liberation, both male and female pronuclei have moved to the center of the egg, forming a fusion nucleus of short duration. One hour after liberation this nucleus is in metaphase or anaphase of the first cleavage.

As the polar bodies are being formed it becomes apparent that the egg is invested in a thin, closely fitting membrane which is now detectable by virtue of being lifted from the surface by the extrusion of the polar bodies.

DISCUSSION

Gonads. From the preceding account it is clear that the yearly turnover in the gonads involves two cyclic processes in opposite phase. One of these is the cycle of development of the sex cells, beginning in the months of June and July and cul-

minating in mature gametes in the following March and April. The other cycle is that of the vasoperitoneal tissue which reaches its ebb at the time of the breeding season and its peak in July and August in the male, or in September and October in the female. The vasoperitoneal tissue thus reaches its greatest development at a time when the gonads are at their smallest, and as the gonad increases in size the vasoperitoneal tissue becomes commensurately reduced. This relationship, together with the intimate contact which exists between the developing gametes and the vasoperitoneal tissue, has led other investigators (Ikeda, 1903; de Selys Longchamps, 1907) to assume that the substance of the vasoperitoneal tissue nourishes the growing germ cells and is therefore exhausted at the time of the ripening of the latter. Ikeda (1903) considers that the cells of the vasoperitoneal tissue act as follicle cells in relation to the gametes, and that the latter absorb nutrient from the vasoperitoneal cells. De Selys Longchamps (1907), however, holds that the spermatocytes and oocytes become free in the body cavity, pass into the cells of the vasoperitoneal tissue and there complete their development. Observations on *Ph. viridis* show that as the ova develop they are covered by a layer of epithelium which disappears, releasing the ova into the body cavity at the time at which the germinal vesicle breaks down. In the case of the male there is no detectable investing membrane and it is possible that the spermatocytes do undergo at least a part of their development in the body cavity. In either case, however, there is no evidence that the germ cells penetrate into the vasoperitoneal cells. It certainly seems possible that the vasoperitoneal tissue nourishes the germ cells in *Ph. viridis,* but the exact nature of the relationship between the two tissues is not clear.

After spawning there remain strands of tissue radiating from the blood caeca and between these strands are the spaces left by the discharge of the ripe gametes. It has been assumed that these strands are the remains of the vasoperitoneal tissue and, in the case of the female, also of the epithelium that once covered the ova. These spaces may be considered either as the now vacant intercellular spaces or, less probably, as the enlarged interiors of vasoperitoneal cells. Later in the year the radiating strands are not prominent and the vacant spaces fill up with new vasoperitoneal tissue. The relationship between the old strands and spaces and the new tissue is uncertain, although it appears that the new tissue gradually forms around the remains of the old. The fully formed vasoperitoneal cells appear to be constructed rather like watery sacs containing globules of various sizes, the nucleus being near the cell membrane. Ikeda (1903) states that in *Ph. australis* and *Ph. ijimai* the vasoperitoneal tissue arises by a proliferation of the peritoneal layer forming the walls of the blood vessels. Cori (1939) says that the situation is similar in *Ph. hippocrepia.* In *Ph. viridis* it has not been possible to determine the origin of this tissue.

Concerning the origin of the reproductive cells, Ikeda's (1903) account for *Ph. ijimai* and *Ph. australis* is in agreement with the findings for *Ph. viridis.* Although oogonia and spermatogonia have only been tentatively identified in *Ph. viridis,* it seems quite certain that they must arise from the peritoneal covering of the blood vessels as described by Ikeda.

Protandry. Apart from the work on the three species which have been shown to be hermaphrodite (*Ph. hippocrepia, Ph. ijimai* and *Ph. australis*) there is very little information concerning the question of protandric hermaphroditism versus

dioecism in this group. De Selys Longchamps, working with *Phoronis psammophila,* found that most individuals contained either testis or ovary, but he obtained one specimen in which the testis surrounded a group of developing oöcytes. On the basis of this evidence *Ph. psammophila* has generally been considered as a protandric hermaphrodite. In several other cases species collected at only one time of the year have shown only ovaries or testes (*Phoronis vancouverensis* and *Phoronopsis harmeri,* Pixel 1912, and *Phoronis mulleri,* de Selys Longchamps 1902). Torrey (1901) found only testes in specimens of *Phoronis pacifica* collected in Humboldt Bay in June, whereas specimens from Puget Sound collected in another year (month not given) contained ova in the nephridia. He suggested that *Ph. pacifica* might be dioecious. Marcus (1949) reports that *Phoronis ovalis* from Brazil contains testes in May and oöcytes in July but it is not known whether or not this species is hermaphrodite.

Brooks and Cowles (1905) have made a more detailed analysis of the cyclic changes in the gonad in *Ph. architecta.* In this species there exists a situation somewhat similar to that described for *Ph. viridis. Ph. architecta* sheds its eggs freely into the water and at the breeding season individual animals contain either testes or ovary, but never both. Male animals possess a lophophoral organ and female animals do not. The males are mature from March to October and the females from May to October. Brooks and Cowles decided that this animal was probably protandric, that the males which were mature in March and April, before the onset of egg laying, had developed from eggs laid early in the previous year, that is, in May or June, and that these male individuals having spawned in March and April of their first year would become females by May. The males which matured in May and later were considered to have developed from eggs laid late in the previous breeding season which had not yet had time to pass through the male phase and become female. The lophophoral organs were found to contain spermatozoa and were considered to act as storage places while the animal changed from the male to the female state. Although spermatozoa were found in the body cavity of females, fertilization was considered to take place in the tentacular crown.

If this hypothesis be applied to *Ph. viridis* several difficulties arise. In the first place there is no lophophoral organ and therefore no storage place for the spermatozoa. In the second place the breeding season for *Ph. viridis* is much shorter; the eggs are shed over a period of only two months as compared with six in the case of *Ph. architecta.* This means that individuals of *Ph. viridis* conceived at the beginning of the egg-laying period could at most be only two instead of six months older than those developing from eggs laid at the end of this period, and such individuals are therefore less likely to have had time to pass through the male phase and become female. Finally it seems that the theory of Brooks and Cowles is, in the case of *Ph. viridis,* a rather roundabout way of explaining a phenomenon which could be a result of a situation in which there was no change of sex during the breeding season, but simply one in which the males matured earlier than the females which would ensure an abundance of spermatozoa when the ova became ripe. The fact that large masses of spermatozoa are found in the nephridia of males suggests that these spermatozoa are shed by the males, and the presence of similar masses in the nephridia and body cavity of females suggests that these masses are collected by the female. The method of this collection is an interesting problem

which might be partially elucidated by a careful study of the currents of the branchial crown and lophophore. It is suggested that the spermatozoa are discharged in compact masses by male animals, some of whom will be no more than a centimeter from the nearest female. The masses of spermatozoa could then be drawn into the tentacular crown of the female by the currents created by the cilia on the tentacles and in some manner passed into nephridia. It is unknown whether the spermatozoa pass into the nephridium by active swimming movements or are swept in by the cilia on the nephridium. The presence of large, compact masses of spermatozoa in the female suggests that they may have been passed inward passively. Considering the probably normal excretory function of the nephridium and the size and position of the nephridiopore, spermatozoan entry is a difficult matter to explain satisfactorily.

Even assuming that the above course of events does take place, there remains the possibility of a change of sex from one year to the next, so that animals which are males one season will be female the following one. If this were so it might also be possible that the spermatozoa seen in the body cavity of the female were left over from the previous male phase and had not entered from the outside. This possibility seems somewhat unlikely since it would necessitate the maintenance of active spermatozoa for a period of eight months, would result in self-fertilization, and would not explain the presence of spermatozoa in the nephridia of both males and females, or the apparent lack of spermatozoa in the reproductive region of females from April to December.

On the basis of present information, therefore, it seems most probable that in *Ph. viridis* the sexes are separate, at least during any one breeding season, and that spermatozoa leave the male and enter the female via the nephridia. Since the evidence is by no means complete, the possibility of protandry cannot be ruled out, but it is at present considered unlikely.

Fertilization. Although spermatozoa have been seen in the body cavity of the female by a number of authors (Brooks and Cowles, 1905; de Selys Longchamps, 1907), they have not been previously described from the nephridium of the female and it has been generally assumed that fertilization took place outside the body, in the tentacular crown. Torrey (1901) and Brooks and Cowles (1905) were certain that the spermatozoa did not penetrate the eggs in the body cavity. Considering the small size of the male nucleus, however, it seems probable that *Ph. viridis* is not unusual and that the presence of two nuclei in the eggs in the body cavity may in the future be demonstrated in other species of phoronids. The fact that all phoronids investigated in this respect are alike in having the first meiotic division of ripe body cavity eggs arrested at metaphase suggests that they may also resemble one another with respect to the site of fertilization. It is not certain when the spermatozoon actually enters the egg of *Ph. viridis,* although it seems very probable that this takes place at the time of the breakdown of the germinal vesicle.

Summary

1. The seasonal changes in the gonad of *Phoronopsis viridis* are described and are seen to involve the proliferation after spawning of vasoperitoneal tissue which is subsequently reduced and largely replaced by developing reproductive tissue.

2. No significant difference between the body lengths of male and female animals was found in the group of 169 animals measured. There is thus no evidence for protandry on the basis of body length.

3. Spermatozoa were found in the nephridia of both male and female animals and in the body cavity of female animals from January to May. Ripe eggs in the body cavity, in which the female nucleus is arrested at first metaphase, also contain a male nucleus. Eggs still covered by the investing epithelium and in which the germinal vesicle is still intact do not contain a male nucleus.

4. *Ph. viridis* is considered to be probably dioecious. Spermatozoa are believed to enter the female through the nephridium and fertilization appears to take place in the body cavity of the female, following the breakdown of the germinal vesicle. The egg when spawned is already fertilized and the maturation divisions proceed as soon as it is shed into sea water.

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